

## IGF-I and IGF-II mRNA expression in slow and fast growing families of USDA103 channel catfish (*Ictalurus punctatus*)

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### Abstract

The objective of this study was to examine insulin-like growth factor (IGF)-I and IGF-II mRNA levels in fast and slow growing families of catfish. Relative levels of IGF-I and IGF-II mRNA were determined by real-time PCR. Family A exhibited a specific growth rate (SGR) of 3.6 and was designated as fast growing, while family H exhibited a SGR of 3.1 and was designated as slow growing ( $P=0.017$ ). Levels of IGF-II mRNA were 3.3-fold greater ( $P=0.006$ ) in muscle for the fast growing family compared to the slow growing family. Levels of IGF-II mRNA were 1.8-fold greater ( $P=0.049$ ) in liver for the fast growing family compared to the slow growing family. Levels of IGF-II mRNA from both fast and slow families were 12.2-fold greater ( $P<0.001$ ) in muscle and 5.8-fold greater ( $P=0.021$ ) in liver, respectively, compared to levels of IGF-I mRNA. Muscle and liver levels of IGF-I mRNA were similar between families. Elevated levels of IGF-II mRNA in muscle and liver compared to IGF-I mRNA, as well as differences in levels of IGF-II mRNA between fast and slow growing families of fish suggests a role of IGF-II in growth of channel catfish.

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**Keywords:** Channel catfish; Gene expression; Real time pcr; Muscle; Liver; IGF-I; IGF-II

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### 1. Introduction

The channel catfish (*Ictalurus punctatus*) is the most extensively cultured food-fish species in North America. Despite its economic significance, there is a lack of information regarding catfish growth as well as mechanisms of action of growth. Attempts to promote growth of catfish using anabolic steroids have not been successful (Simone, 1990; Gannam and Lovell, 1991; Davis et al., 2000) and influence of exogenous growth hormone (GH) administration on catfish growth has been inconsistent (Wilson et al., 1988; Silverstein et al., 2000; Peterson et al., 2004), raising questions about the physiological role of GH in catfish. Wilson et al. (1988) injected fingerling channel catfish, *I. punctatus* (strain of catfish not published), with recombinant bovine growth hormone (rbGH) and showed an increase in

growth rate when compared to placebo-injected controls. Silverstein et al. (2000) injected Norris and USDA103 strains of channel catfish with rbGH at two different temperatures, 26.0 and 21.7 °C, and observed that growth rate was increased only in the USDA103 catfish at 26.0 °C, while it was increased in both strains at 21.7 °C compared to controls. Peterson et al. (2004) injected Norris and USDA103 with rbGH at three doses (30, 60 and 120 µg/g body weight) every 3 weeks for 9 weeks and found that all doses increased final weights in USDA103 catfish while only the high dose increased final weights in the Norris catfish compared to controls. Overall, these studies suggest that exogenous GH can increase growth rates in channel catfish but strain, temperature and dose of GH influences its effect.

Tang et al. (2001) exposed catfish to brackish water and showed that pituitary GH mRNA increased, suggesting that GH may play a role in hypoosmoregulation. In contrast, Eckert et al. (2001) injected catfish with ovine GH prior to exposure to brackish water and could not demonstrate any

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hypoosmoregulatory actions of GH. It is evident that the roles of GH in osmoregulation and growth of channel catfish are not well understood.

Mammalian growth is primarily controlled by the GH-insulin-like growth factor-I (IGF-I) axis (Daughaday and Rotwein, 1989; Baxter, 1994). IGF-I functions as a postnatal GH-dependent growth factor, while insulin-like growth factor-II (IGF-II) shows little dependence on GH and is considered a major fetal growth factor. GH regulates the expression of IGF-I, while IGF-specific binding proteins (IGFBPs) regulate IGF activity. In mammals, the IGFBPs are composed of a family of six proteins (IGFBP-1 to -6) that bind to IGFs with high affinity and specificity (Ferry et al., 1999) and play a central role in coordinating and transporting IGFs to target cells (Baxter, 1994).

The GH-IGF axis also plays a major role in the endocrine control of fish growth. Growth is primarily controlled by the availability of GH, IGF-I and IGF-II, and their respective receptors, and IGFBPs (Peter and Marchant, 1995; Le Bail et al., 1998; Moriyama et al., 2000). The presence of IGFBPs has been demonstrated in several teleost species (reviewed by Kelley et al., 2002) including catfish (Delahunty et al., 1993; Johnson et al., 2003; Peterson et al., 2004; Peterson and Small, 2004). Growth hormone is a potent regulator of liver IGF-I expression in several species of fish (Duan et al., 1995; Kajimura et al., 2001; Biga et al., 2004; Pierce et al., 2004) and GH can also regulate the IGF-II gene in rainbow trout (*Oncorhynchus mykiss*) (Shamblott and Chen, 1999). Using primary cell cultured hepatocytes of tilapia, Schmid et al. (2000) showed that treatment with GH at concentrations of 0.1 nM to 1  $\mu$ M caused a dose-dependent increase in the amount of IGF-I mRNA. In addition, Silverstein et al. (2000) observed a slight but significant increase in plasma levels of IGF-I in USDA103 and Norris strains of catfish injected with recombinant bovine growth hormone.

To our knowledge, there is no information regarding the role of IGF-II in channel catfish. IGF-II gene expression has been demonstrated in a variety of extrahepatic tissues of fish (Shamblott and Chen, 1992; Duguay et al., 1996; Collet et al., 1997; Loffing-Cueni et al., 1999; Schmid et al., 1999; Degger et al., 2001; Tse et al., 2002; Radaelli et al., 2003). Maures et al. (2002) observed the presence IGF-II mRNA throughout embryogenesis in zebra fish (*Danio rerio*). Radaelli et al. (2003) demonstrated that the IGF-II gene is expressed at high levels from the early stages of embryonic development until the adult stage in gilthead seabream (*Sparus aurata*). Chauvigne et al. (2003) observed an increase in IGF-II mRNA levels in rainbow trout during refeeding. This study suggested a role for IGF-II in promoting muscle compensatory growth induced by refeeding. In another study, Gabillard et al. (2003) reported that the level of IGF-II mRNA in muscle was not correlated to temperature-associated differences in rainbow trout growth. Although the role of IGF-II in fish growth is not clearly defined, these studies suggest that IGF-II may be involved in regulating growth and development of some fish species.

Full-sib catfish families exhibiting fast and slow growth are useful for studying the regulatory roles of genes and gene products of the IGF system. Furthermore, differences in gene products may help explain differences in growth. The present study was conducted to test the hypothesis that differences in levels of IGF-I or IGF-II mRNA could be correlated to differences in growth rates among fast and slow growing families of catfish.

## 2. Materials and methods

### 2.1. Catfish families

All fish used in the study were from natural pond spawns and were reared in indoor tanks in a common environment at the USDA-ARS Catfish Genetics Research Unit, Stoneville, MS. Families were selected from approximately 100 full-sib families of the USDA103 strain that had undergone a 30-day growth study at 100 days post-hatch. The four fastest and the four slowest growing families were selected based on the 30-day growth data.

Prior to randomization into tanks, approximately 100 fish from each of the eight selected families were placed into separate 120-l holding tanks for 1 day. Forty catfish averaging 18.1 g from each of the eight families (total  $n=320$ ) were then randomly assigned to four 76-l tanks (10 fish/tank) and allowed to acclimate for 7 days. The fish were fed once per day to apparent satiation and reared in 26.0 °C flow-through well water and a 14:10-h L/D photoperiod. A commercial 36% crude protein floating catfish feed (Land O'Lakes Farmland Feed, Fort Dodge, IA, USA) was used throughout the study. Water quality (pH~8.5 and dissolved oxygen levels >5.0 mg/l) and flow rates were similar between tanks. The fish were maintained for 6 weeks and the amount of feed was recorded weekly. Specific growth rates (SGR) were calculated from the formula  $((\ln(WT) - \ln(wt)) / (T - t)) \times 100$  where WT and wt are initial and final weights, respectively, and T and t are initial and final times (weeks), respectively. Feed conversion ratios (FCR) were calculated as ingested food (g)/weight increase (g).

### 2.2. RNA purification

After the 6-week growth study, fish were weighed as a group and 3 fish from each tank (12/family) were euthanized with an overdose (0.3 g/l) of tricaine methanesulfonate (Finquill; Argent Chemical Laboratories, Redmond, WA). A transverse slice of fast muscle (approximately 100 mg) located beneath the dorsal fin was taken for RNA extraction. Approximately 100 mg of liver was also taken for RNA extraction. Samples were immediately placed in 1 ml RNA later™ (Ambion, Austin, TX, USA) and kept at 4 °C for 2–4 days until RNA from all samples was isolated. Total RNA was isolated using an AquaPure RNA Isolation kit (BioRad, Hercules, CA, USA). The integrity of the RNA was verified

by visualization of the 18S and 28S ribosomal bands stained with ethidium bromide after electrophoresis on 2.0% agarose gels.

### 2.3. Plasmid standards

A PCR fragment was generated using primers listed in Table 1 and was cloned into the pCR®4-TOPO vector (Invitrogen, Carlsbad, CA, USA) and introduced into One Shot® TOP10 Chemically Competent *Escherichia coli* (Invitrogen) cells. The identity of the cloned inserts was confirmed by sequencing at the USDA Mid-South-Area Genomic Laboratory, Stoneville, MS. The DNA concentration of each resulting recombinant plasmid was measured using the NanoDrop ND-100 spectrophotometer (NanoDrop Technologies, Rockland, DE). The standard curve for each gene was generated from two replicates of 10-fold serial dilutions of recombinant plasmid (8 points). Amplification products were quantified by comparison of experimental Ct (threshold cycle-defined as the PCR cycle where an increase in fluorescence first occurred) levels with those of a standard curve.

### 2.4. Real-time PCR

Samples were analyzed only in the slowest growing (H) and fastest growing (A) families of fish. Total RNA (1 µg)

Table 1  
Nucleotide sequences of the PCR primers and probes used to assay gene expression by real-time quantitative PCR

Gene	Primer	Sequence	PCR product length (nt)
IGF-I	Sense	CTG TGA GCT GAA ACG ACT CG	124
	Antisense	CCA GAT ATA GGT TTT CTT TGG TG	
	Probe <sup>a</sup>	CGA GAG CAA CGG CAC ACA GAC ACG C	
IGF-II	Sense	GCG AGG ACA GAG GCT TCT AC	140
	Antisense	CTC TGA CTT GGC TGG CTT GG	
	Probe <sup>a</sup>	TCT TCC ACA ATG CCA CGG TTC TGA G	
18S	Sense	GAG AAA CGG CTA CCA CAT CC	128
	Antisense	GAT ACG CTC ATT CCG ATT ACA G	
	Probe <sup>b</sup>	GGT AAT TTG CGC GCC TGC TGC C	

<sup>a</sup> The probes IGF-I (GenBank Accession No. AY353852) and IGF-II (GenBank Accession No. AY615885) were dual labeled with a reporter dye (FAM, 6-carboxyfluorescein) at the 5' end and a quencher dye (BHQ-1, Black Hole quencher-1) at the 3' end (Biosearch Technologies, Novato, CA).

<sup>b</sup> The probe 18S (GenBank Accession No. AF021880) was dual labeled with a reporter dye Texas red (sulforhodamine 101) at the 5' end of the probe and a quencher dye (BHQ-1) at the 3' end (Biosearch Technologies).

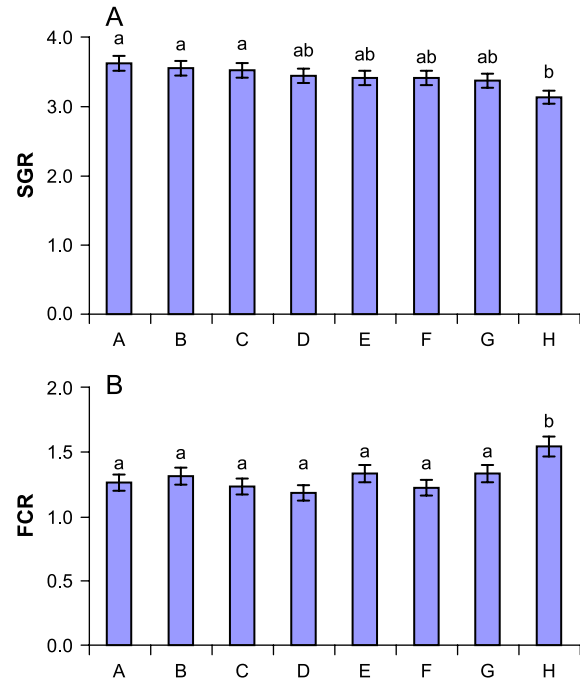


Fig. 1. (A) SGR of eight families of USDA103 channel catfish. SGR was calculated  $((\ln(WT) - \ln(wt))/(T - t)) \times 100$  where WT and wt are initial and final weights, respectively, and T and t are initial and final times (weeks). (B) FCR of eight families of USDA103 channel catfish. FCR was calculated as ingested food (g)/weight increase (g). Values are expressed as means  $\pm$  standard error of the mean. Families with different superscripts are significantly different ( $P < 0.05$ ).

from liver and muscle was reverse-transcribed in 10 µl reactions using the iScript cDNA Synthesis Kit (BioRad). After the RNA was reverse-transcribed, the cDNA was quantified using the NanoDrop ND-100 spectrophotometer. Real-time PCR was performed using the iCycler iQ™ (BioRad). The primers, probes and accession numbers for IGF-I, IGF-II and 18S are listed in Table 1. Primer and probe sequences were designed with Beacon Designer 2.0 (Premier BioSoft) software. For the IGF-I gene, the sense primer was in exon 3 and the antisense primer spanned exons 3 and 4. For the IGF-II gene, the sense primer was in exon 1 and the antisense primer was in exon 2. In order to avoid amplification of the target gene in genomic DNA, the probe for IGF-I and IGF-II spanned the junction between two exons, covered by the forward and reverse primers.

Each amplification reaction mixture (12.5 µl) contained 300 ng of cDNA; 1× iQ™ Supermix (Bio-Rad Laboratories, Hercules, CA) which consisted of: 10 mM KCl, 4 mM Tris-HCl, pH 8.4, 0.16 mM dNTPs, 5 U/ml iTaq polymerase, 0.6 mM MgCl<sub>2</sub>, and stabilizers; 10 µM (IGF-I, IGF-II or 18S) of each primer; and dual-labeled probe (5 µM of IGF-I or IGF-II; 1 µM of 18S). The real-time PCR protocol for IGF-I, IGF-II and 18S was 3 min at 95 °C, 45 cycles of 95 °C-15 s, 60 °C-1 min. PCR efficiencies of all reactions were between 90% and 100%. All measurements were performed in triplicate. All specific quantities were normalized against the amount of 18S rRNA amplified. No strain

effect was seen on the 18S values. In determining a normalized IGF-I or IGF-II value for each sample, the amount of target molecule was divided by the amount of endogenous control (18S). Hence, all calculated concentrations are relative to the concentration of the standard and expressed in arbitrary units.

### 2.5. Statistical analysis

Statistically significant differences ( $P<0.05$ ) in tissue gene expression levels, SGR and FCR were detected using ANOVA (Statistical Analysis System Version 9.0 software, SAS Institute, Cary, NC, USA) followed by a Duncan's multiple range test. Tank served as the experimental unit for each variable measured.

## 3. Results

### 3.1. Growth rate and feed efficiency

Family H exhibited a lower ( $P<0.05$ ) SGR compared to families A, B and C and had the poorest FCR ( $P<0.01$ ) compared to the other catfish families (Fig. 1). Family H also had the lowest SGR of the eight families during the

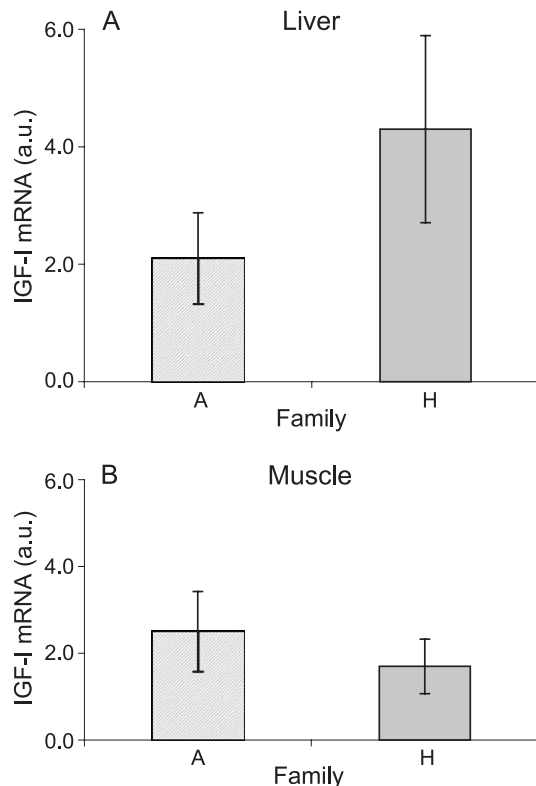


Fig. 2. (A) Liver IGF-I mRNA levels in fast (A) and slow (H) growing families of catfish. (B) Muscle IGF-I mRNA levels in fast (A) and slow (H) growing families of catfish.  $N=12$  per family. The 18S rRNA measurements served as an internal control and were used to normalize the IGF-I expression levels (a.u.=arbitrary units). Standard error bars represent standard error of the mean ( $P>0.10$ ).

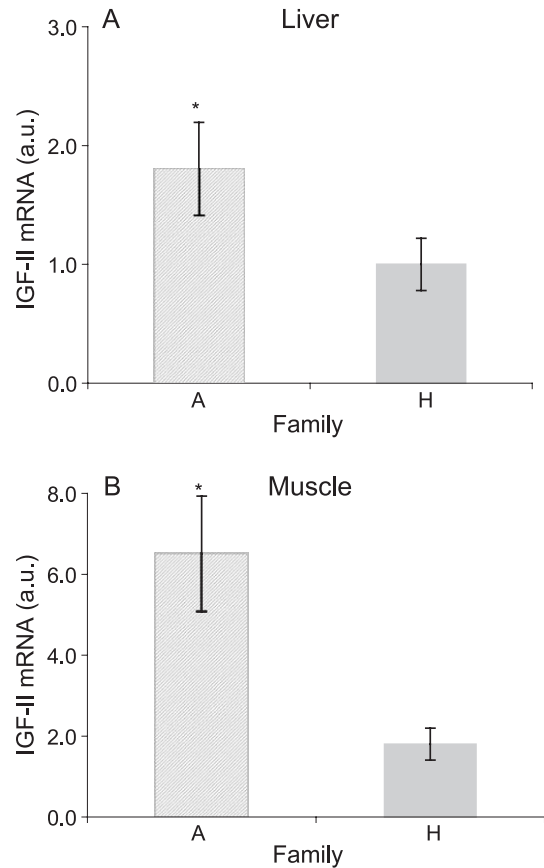


Fig. 3. (A) Liver IGF-II mRNA levels in fast (A) and slow (H) growing families of catfish. (B) Muscle IGF-II mRNA levels in fast (A) and slow (H) growing families of catfish.  $N=12$  per family. The 18S rRNA measurements served as an internal control and were used to normalize the IGF-II expression levels (a.u.=arbitrary units). Standard error bars represent standard error of the mean and significant differences ( $P<0.05$ ) denoted by an asterisk.

previous 30-day growth study (data not shown). Family A exhibited the highest SGR ( $P=0.017$ ) (3.6 vs. 3.1) than family H (Fig. 1) during the 6-week study but was not different from families B, C, D, E, F and G. Family A also had the highest SGR during the previous 30-day growth study (data not shown). Because Family A had the highest SGR during the previous 30-day study as well as one of the highest SGR in the 6-week study, it was designated as the “fast” growing family. Similarly, because family H had the lowest SGR during the 30-day study as well as the lowest SGR in the 6-week study, it was designated as the “slow” growing family. These results confirm growth rate and feed efficiency differences among USDA103 channel catfish families.

### 3.2. Levels of IGF-I and IGF-II mRNA in muscle and liver

Levels of IGF-I mRNA were not different ( $P>0.10$ ) in the muscle and liver of the fast and slowing growing fish, respectively (Fig. 2A and B). However, levels of IGF-II mRNA were 1.8-fold greater ( $P<0.05$ ) in the liver of fast



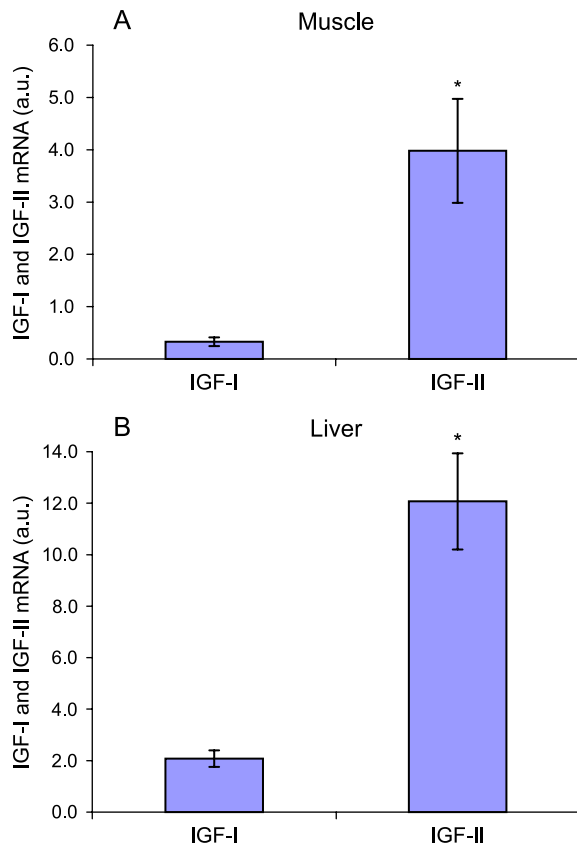


Fig. 4. (A) Comparison of muscle IGF-II mRNA to muscle IGF-I in all families of catfish. (B) Comparison of liver IGF-II mRNA to liver IGF-I in all families of catfish.  $N=24$  per tissue. The 18S rRNA measurements served as an internal control and were used to normalize the IGF-I and IGF-II expression levels (a.u.=arbitrary units). Standard error bars represent standard error of the mean and significant differences ( $P<0.05$ ) denoted by an asterisk.

growing fish (A) relative to slow growing fish (H) (Fig. 3A). Levels of IGF-II mRNA were 3.3-fold greater ( $P<0.01$ ) in the muscle of fast growing fish (A) relative to slow growing fish (H) (Fig. 3B). Levels of IGF-II mRNA were 12.2-fold greater ( $P<0.001$ ) than IGF-I mRNA in the muscle (Fig. 4A), and IGF-II mRNA levels were 5.8-fold greater ( $P=0.021$ ) compared to IGF-I mRNA levels in liver (Fig. 4B). The higher IGF-II mRNA levels observed in the muscle and liver compared to IGF-I mRNA levels were observed similarly in fast and slow growing fish.

#### 4. Discussion

To predict how biological systems function during growth, it is necessary to understand the mechanisms that control them. Unfortunately with channel catfish, little is known about growth regulatory mechanisms. The objective of the present study was to compare components of the IGF system between fast growing and slow growing channel catfish and to gain a better understanding of the roles of these components in growth of catfish. Toward this goal, we

developed a real-time PCR assay to measure levels of IGF-I and IGF-II mRNA. Fast and slow growing fish used in this study were selected from approximately 100 full-sib families that had previously undergone a 30-day growth study. Results of the prior 30-day growth study were similar to the results of the present growth study. These experiments demonstrated differences in growth rate and feed efficiency among families of USDA103 channel catfish.

Levels of IGF-I and IGF-II mRNA were measured in both the muscle and liver of experimental fish. The liver appears to be the organ with the highest level of IGF mRNA production, with levels of IGF-I and IGF-II mRNA at least three times higher than that of muscle (Figs. 3 and 4). Vong et al. (2003) and Caelers et al. (2004) also reported the liver as the major organ expressing both IGF-I and IGF-II mRNA in common carp (*Cyprinus carpio*) and tilapia (*Oreochromis niloticus*), respectively. Levels of IGF-I mRNA were similar in the muscle and liver of the fast and slow growing fish. The lack of difference in hepatic expression of IGF-I was surprising since faster growth was presumably being mediated by IGF-I. However, this does not imply that IGF-I has no function in growth of channel catfish. The fact that muscle and liver IGF-I mRNA levels were not related to the growth rate of the faster growing fish only means that muscle and liver IGF-I mRNA cannot explain differences in growth between the two families.

It is possible that IGF-I produced by other tissues play a significant role in the growth of channel catfish. Studies in other species of fish have reported expression of IGF-I in the brain, gill, heart, intestine, kidney, spleen and testes (Vong et al., 2003; Biga et al., 2004; Caelers et al., 2004). IGF-I may have an important autocrine/paracrine role in catfish. In support of an important autocrine/paracrine IGF-I role, mice that have had the IGF-I gene deleted in the liver have shown that liver IGF-I is not essential for normal growth and development (Yakar et al., 1999; Sjogren et al., 1999). These studies provide evidence that autocrine/paracrine IGF-I can support normal post-natal growth and development.

Only one study has measured plasma levels of IGF-I in channel catfish. Silverstein et al. (2000) observed a small but significant increase in circulating levels of IGF-I in strains of USDA103 and Norris catfish injected with recombinant bovine growth hormone. In the Norris strain, plasma IGF-I levels increased from 5.39 to 8.81 ng/ml and, in the USDA103 strain, 7.76 to 12.03 ng/ml (Silverstein et al., 2000). More research is needed on the relationship between growth and protein and expression levels of IGF-I in channel catfish. Efforts into the development of homologous protein assays for IGF-I have been hampered by the lack of native or recombinant catfish IGF-I needed to produce catfish specific IGF-I antibody.

In mammals, IGF-II mRNA is detected in many fetal tissues, but decreases quickly during postnatal development (Daughaday and Rotwein, 1989). In contrast, teleostean tissues express substantial amounts of IGF-II later in life (Gabillard et al., 2003; Chauvigne et al., 2003; Radaelli et

al., 2003; Vong et al., 2003; Caelers et al., 2004). Vong et al. (2003) reported that IGF-II mRNA levels were higher than IGF-I mRNA levels in extrahepatic tissues such as the brain, heart, intestine, kidney and muscle. In tilapia, Caelers et al. (2004) also reported higher IGF-II mRNA levels in the heart, gut, kidney, muscle and spleen compared to IGF-I mRNA levels. Caelers et al. (2003) also demonstrated the presence of IGF-II mRNA in numerous neurons in adult fish brain while Schmid et al. (1999) and Perrot et al. (2000) have demonstrated IGF-II mRNA in parenchymal cells of the fish ovary.

We found that IGF-II mRNA levels in liver and muscle was higher than IGF-I mRNA levels. This is the first report of IGF-II mRNA levels being higher in the liver than IGF-I mRNA levels in any species. In addition, we found that levels of IGF-II mRNA was greater in muscle and liver of fast growing fish compared to slow growing fish. These results suggest a role of IGF-II in channel catfish growth. Support for a role of IGF-II in growth stems from research with the pig. Van Laere et al. (2003) identified an IGF-II mutation as a quantitative trait loci affecting postnatal muscle growth in swine and suggested that exploiting this IGF-II polymorphism may prove useful in selecting pigs for faster growth. As such, polymorphism typing of IGF-II in channel catfish may also be worth investigating.

In conclusion, we have developed a highly sensitive method of measuring IGF-I and IGF-II mRNA levels in catfish tissues by real-time PCR. The elevated levels of IGF-II mRNA in the muscle and liver compared to IGF-I mRNA, as well as differences in levels of IGF-II mRNA, between fast and slow growing families of fish provide evidence for a role of IGF-II in catfish growth. This research suggests that the IGF system may be useful for identifying USDA103 families that have greater growth potential for selective breeding.

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